v-ATPase is a key player in lipid-induced cardiomyopathy

Valorization

Social and clinical relevance

Diabetes has now reached epidemic proportions in the world range. Today, 350 million people across the world are living with diabetes and 90% of the burden is caused by type 2 diabetes. The American National Diabetes Statistics has reported that in the U.S.A. the total prevalence is 29.1 million children and adults, which represents 9.3% of the population. It is believed that 7/10 of those people have been diagnosed, whereas the remaining people are still undiagnosed. In the Netherlands, one million people suffer from type 2 diabetes. Every year, the amount of diabetic patients increases by 70,000.

Annually there are 4 million deaths due to diabetes in the world. The major cause of mortality in type 2 diabetes is cardiovascular disease. There is increasing evidence that type 2 diabetes is associated with cardiomyopathy, independent of hypertension and coronary artery disease. Furthermore, cardiac insulin resistance and metabolic alterations, especially lipid accumulation in cardiomyocytes (lipotoxicity) are primary causes leading to cardiac dysfunction. In a broader perspective, there is growing awareness that lipotoxicity in non-adipose tissues such as myocardium is a common denominator of pathology in three inter-related chronic conditions, i.e., type 2 diabetes, obesity and the metabolic syndrome, which together are becoming a major health problem. As a result, the search for new avenues for therapy of lipotoxicity is of great importance for illnesses directly and indirectly affecting proper cardiac functioning.

Clinical research in diabetic humans is to a large extent confined to non-invasive methods, which limit the study of the molecular basis of type 2 diabetes and its complications (i.e., lipid-induced cardiomyopathy). The successful results obtained in this thesis, also including experiments with human cardiomyocytes, highlight a novel molecular target to prevent lipid accumulation, preserve insulin sensitivity, and eventually restore cardiac function in the lipid-overloaded heart. Considering the high occurrence of type 2 diabetes and diabetic cardiomyopathy, our findings hold significant promise to the clinical application and therefore eventually may alleviate the economic burden of the society.

Novelty of the concept

Current antidiabetic treatments (i.e., biguanides, glucosidase inhibitors, and thiazolidinediones) in humans are only modestly effective, and most of these treatments show complications. A number of studies have indicated some novel pharmaceutical targets with different underlying pharmacological mechanisms. Unfortunately, for none of those targets as new-mechanism based drug has yet reached the patients, mainly due to

off-target effects.

Already in the nineties, the research group as a part of which I performed my PhD research, has identified CD36 as the main fatty acid transporter in the heart. Upon lipid oversupply, increased CD36-mediated fatty acid uptake is the key process resulting in cardiac lipid accumulation, and subsequent insulin resistance and contractile dysfunction. Hence, a pharmacological blockade of CD36 could theoretically prevent the maladaptive lipid-induced alterations during lipid oversupply. However, CD36 is not expected to be a suitable drug target, because it has multiple functions not only related to long-chain fatty acid (LCFA) uptake in multiple tissues. Alternatively, given that increased CD36-mediated fatty acid uptake is due to increased CD36 translocation, the CD36 translocation machinery (consisting of over 50 proteins, in analogy to the GLUT4 translocation machinery) could provide further drug targets to limit fatty acid uptake in the lipid-overloaded heart. Yet, the CD36 translocation machinery has remained almost completely unidentified. Moreover, how lipids stimulate CD36 translocation to the sarcolemma was still entirely unknown. However, in this thesis we discovered that as part of the CD36 translocation machinery, the endosomal proton pump, also known as vacuolar-type H^+-ATPase (v-ATPase) is a key player in regulation of CD36 translocation. More specifically, v-ATPase becomes inhibited during lipid oversupply, which then directly leads to insulin resistance and contractile dysfunction. The potential underlying mechanism of lipid-induced v-ATPase inhibition is further disclosed, and was observed to involve the disassembly of the two sub-complexes of v-ATPase. Hence, strategies to induce re-assembly of v-ATPase would result in v-ATPase re-activation and reversal of the subsequent maladaptive lipid-induced alterations. Indeed, pioneering studies described in **chapter 5** have already shown that high glucose concentrations induce the re-assembly of v-ATPase. Yet, it seems rather counterintuitive that high glucose infusion would be a part of a novel strategy to combat insulin resistance in the lipid-overloaded diabetic heart. Perhaps, other therapeutic strategies that would selectively upregulate glucose uptake and/or generate intracellularly the acting glucose intermediate would be better suited to re-activate v-ATPase.

Potential application

V-ATPase is ubiquitously present in mammalian tissues. Consequently, a lack of selectivity for the heart could be a serious barrier to use v-ATPase as drug target. Importantly, v-ATPase has multiple subunits, i.e., at least 14. Several of these subunits are expressed in different isoforms in different tissues. Therefore, variations in the sensitivity of these isoforms towards drugs might provide an attractive platform for cardiac-specific targeting of v-ATPase. Notwithstanding, it is important to realize that the onset of cardiac insulin resistance goes hand in hand with skeletal muscle and liver insulin resistance.

Hence, simultaneous re-activation of v-ATPase in these latter tissues, if the same mechanisms of v-ATPase regulation would apply, presumably would be beneficial as well.

In summary, v-ATPase is a promising and innovative target against type 2 diabetes and diabetic cardiomyopathy, even though a long road still awaits its validation, drug discovery, development and clinical testing, before introducing a new medication into the market. Further studies are now ongoing to overexpress GLUT1, the constitutive glucose transporter, or PKD1, a key signaling kinase with a specific involvement in translocation of the main cardiac glucose transporter GLUT4, to increase cardiac glucose uptake. Overexpression of these proteins in lipid-overexposed cardiomyocytes is expected to lead to v-ATPase re-assembly, and could provide further proof-of-principle for v-ATPase re-assembly as anti-diabetic strategy.